Amendments to the Specification

Please amend the specification as follows:

Please replace the title of the invention with the following new title:

~~METHOD FOR SCREENING COMPOUNDS THAT INHIBIT LYSOPHOPHATIDIC ACID ACYLTRANSFERASE~~ HUMAN LYSOPHOSPHATIDIC ACID ACYLTRANSFERASE GAMMA-1 POLYPEPTIDE

Please replace the first paragraph under the application title with the following rewritten paragraph:

This is a Divisional Application of Application No. 09/970,989, filed on 10/05/2001, now U.S. Patent No. 6,670,143, which is a Divisional Application of Application No. 09/215,252, filed on December ~~12~~ 18, 1998, now U.S. Patent No. 6,300,487, which is a Continuation-in–Part of Application No. 08/618,651, filed March 19, 1996, now U.S. Patent No. 6,136,964.

Please replace the paragraphs beginning at page 5, line 9, through page 6, line 2, with the following rewritten paragraphs:

Figure 1 shows the DNA sequence (SEQ ID NO: 1) of the cDNA insert of pZplat.11 encoding hLPAATα (SEQ ID NO: 2).

Figure 2 shows amino acid sequence alignment of the human LPAATα (SEQ ID NO: 2) coding sequence, the yeast LPAAT (SEQ ID NO: 3) coding sequence, *E. coli* LPAAT (SEQ ID NO: 4) coding sequence, and the maize LPAAT (SEQ ID NO: 5) coding sequence. This comparison shows that human LPAATα has the greatest extended homology with yeast or *E. coli* LPAAT than with the plant LPAAT.

Figure 3 shows the DNA sequence (SEQ ID NO: 6) of the cDNA insert pSP.LPAT3 encoding hLPAATβ. The nucleotide sequence analysis and restriction mapping of the cDNA

clone revealed a 5' untranslated region of 39 base pairs and an open reading frame encoding a 278 amino acid polypeptide that spans positions 40-876. It also shows a 3' untranslated region of 480 base pairs from pSP.LPAT3. The initiation site for translation was localized at nucleotide positions 40-42 and fulfilled the requirement for an adequate initiation site (Kozak, *Critical Rev. Biochem. Mol. Biol.* 27:385-402, 1992).

Figure 4 shows the sequence of the hLPAATβ 278 <u>(SEQ ID NOS: 6 & 7)</u> amino acid open reading frame. The amino acid sequence was used as the query sequence to search for homologous sequences in protein databases. Search of the database based on Genbank Release 92 from the National Center for Biotechnology Information (NCBI) using the blastp program showed that this protein was most homologous to the yeast, bacterial and plant LPAATs.

Figure 5 shows amino acid sequences alignment of this putative human LPAATβ <u>(SEQ ID NO: 7)</u> coding sequence, human LPAATα <u>(SEQ ID NO: 2)</u> coding, the yeast LPAAT <u>(SEQ ID NO: 3)</u> coding sequence, the bacterial (*E. coli* <u>(SEQ ID NO: 4)</u>, *H. influenzae* <u>(SEQ ID NO: 8)</u>, and *S. typhimurium* <u>(SEQ ID NO: 9)</u>) LPAAT coding sequences, and the plant (*L. douglassi* <u>(SEQ ID NO: 10)</u> and *C. nucifera* <u>(SEQ ID NO: 11)</u>) LPAAT coding sequences, revealing that the human LPAAT coding sequences have a much more extended homology with the yeast or the bacterial LPAAT than with the plant LPAAT.

Please replace the paragraphs at page 6, lines 12-16, with the following rewritten paragraphs:

Figure 9 shows the DNA <u>(SEQ ID NO: 12)</u> and the translated sequence of LPAATУ1 (SEQ ID NO: 13).

Figure 10 shows the DNA <u>(SEQ ID NO: 14)</u> and the translated sequence of LPAATγ2 (SEQ ID NO: 15).

Figure 11 shows the DNA <u>(SEQ ID NO: 16)</u> and the translated sequence of LPAAT (SEQ ID NO: 17).

Figure 12 shows the LPAAT amino acid sequence alignment for human LPAAT $\gamma 1$ (SEQ ID NO: 13), $\gamma 2$ (SEQ ID NO: 15) and δ (SEQ ID NO: 17).

Please replace the paragraph on page 13, lines 15-32, with the following rewritten paragraph:

The polynucleotides of the present invention can be inserted into an expression vector by standard subcloning techniques. In a preferred embodiment, an *E. coli* expression vector is used which produces the recombinant protein as a fusion protein, allowing rapid affinity purification of the protein. Examples of such fusion protein expression systems are the glutathione S-transferase system (Pharmacia, Piscataway, NJ), the maltose binding protein system (NEB, Beverley, MA), the thiofusion system (Invitrogen, San Diego, CA), the Strep-tag II system (Genosys, Woodlands, TX), the FLAG system (IBI, New Haven, CT), and the 6xHis (SEQ ID NO: 43) system (Qiagen, Chatsworth, CA). Some of these systems produce recombinant polypeptides bearing only a small number of additional amino acids, which are unlikely to affect the LPAAT ability of the recombinant polypeptide. For example, both the FLAG system and the 6xHis (SEQ ID NO: 43) system add only short sequences, both of which are known to be poorly antigenic and which do not adversely affect folding of the polypeptide to its native conformation. Other fusion systems produce proteins where it is desirable to excise the fusion partner from the desired protein. In a preferred embodiment, the fusion partner is linked to the recombinant polypeptide by a peptide sequence containing a specific recognition sequence for a protease. Examples of suitable sequences are those recognized by the Tobacco Etch Virus protease (Life Technologies, Gaithersburg, MD) or Factor Xa (New England Biolabs, Beverley, MA) or enterokinase (Invitrogen, San Diego, CA).

Please replace the paragraphs beginning on page 20, line 23, through page 21, line 13, with the following rewritten paragraphs:

Search of the Genbank database of expressed sequence *tag* (dbest) using either the yeast or plant LPAAT protein sequences as probe came up with several short stretches of cDNA sequences with homology to the yeast or plant LPAAT protein sequence. These cDNA sequences of interest were derived from single-run partial sequencing of random human cDNA clones projects carried out by either the WashU-Merck EST or the Genexpress-Genethon program. An example of the amino acids sequence homology between the yeast LPAAT and a human cDNA clone (dbest#102250) is shown below by comparing SEQ ID NO. [[3]] 18 (top amino acid sequence) with SEQ ID NO [[4]] 19 (bottom amino acid sequence):

```
PFKKGAFHLAQQGKIPIVPVVVSNTSTLVSPKYGVFNRGCMIVRILKPISTE
*   ****** *   **** * *            *  * * ** * *  **
PSNCGAFHLAVQAQVPIVPIVMSSYQDFYCKKERRFTSGQCQVRVLPPVPTE
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The top line refers to the yeast LPAAT sequence from amino acids 169 to 220 and the bottom line refers to the homologous region from the dbest clone#102250. Identical amino acids between these two sequences are shown in block letters with asterisks in between.

Accordingly, a synthetic oligonucleotide (o.BLPAT.2R), 5'-TGCAAGATGGAAGGCGCC-3' (SEQ ID NO. [[5]] 20), was made based on the complement sequence of the conserved amino acids region, GAFHLA (SEQ ID NO. [[6]] 21), of clone#102250. o.BPLAT.2R was radiolabeled at its 5'-end using γ-^{32}P-ATP and T4 polynucleotide kinase as a probe in screening a λzap human brain cDNA library (Stratagene).

Please replace the paragraph on page 23, lines 9-17, with the following rewritten paragraph:

The top line refers to the yeast LPAAT sequence from amino acids 171 to 230 (SEQ ID NO. [[9]] 22) and the bottom line refers to the homologous region from the dbest clone#363498 using the +1 reading frame (SEQ ID NO. [[10]] 23). Identical and conserved

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amino acids between these two sequences are shown with double dots and single dot, respectively, in between. In order to find out if such cDNA clones with limited homology to yeast LPAAT sequence indeed encode human LPAATβ sequence, it was necessary to isolate the full-length cDNA clone, insert it into an expression vector, and to test if cells transformed or transfected with the cDNA expression vector produced more LPAAT activity.

Please replace the paragraph beginning on page 23, line 18, through page 24, line 4, following rewritten paragraph:

Accordingly, two synthetic oligonucleotides, 5'-CCTCAAAGTGTGGATCTATC-3' (o.LPAT3.F) (SEQ ID NO. [[11]] 24) and 5'-GGAAGAGTACACCACGGGGA C-3' (o.LPAT3.R), (SEQ ID NO. [[12]] 25) were ordered (Life Technologies, Gaithersburg, MD) based on, respectively, the coding and the complement sequence of clone#363498. o.LPAT3.R was used in combination with a forward vector primer (o.sport.1), 5'-GACTCTAGCC TAGGCTTTTG C-3'(SEQ ID NO. [[13]] 26) for amplification of the 5'-region, while o.LPAT3.F was used in combination with a reverse vector primer (o.sport.1R), 5'-CTAGCTTATA ATACGACTCA C-3' (SEQ ID NO. [[14]] 27), for amplification of the 3'-region of potential LPAATβ sequences from a pCMV.SPORT human leukocyte cDNA library (Life Technologies, Gaithersburg, MD). A 700 bp PCR fragment derived from o.sport.1 and o.LPAT3.R amplification was cut with *EcoR* I before inserting in between the *Sma* I and *EcoR* I of pBluescript(II)SK(-) (Stratagene, LaJolla, CA) to generate pLPAT3.5'. A 900 bp PCR fragment derived from o.sport.1R and o.LPAT3.F amplification was cut with *Xba* I before inserting in between the *Sma* I and *Xba* I of pBluescript(II)SK(-) (Stratagene, LaJolla, CA) to generate pLPAT3.3'. Nucleotide sequencing analysis of the cDNA inserts from these two plasmids showed they contained overlapping sequences with each other, sequences that matched with the dbEST#363498 as well as extensive homology with the yeast LPAAT amino acids sequence (Nagiec et al., *J. Biol. Chem.* 268:22156-22163, 1993). To assemble the two halves of the cDNA into a full-length clone, the 560 bp *Nco* I - *Nar* I fragment from pLPAT3.5' and the 780 bp *Nar* I - *Xba* I fragment from pLPAT3.3' were

inserted into the *Nco* I / *Xba* I vector prepared from pSP-luc+ (Promega, Madison, WI) via a three-part ligation to generate pSP.LPAT3.

Please replace the paragraph beginning on page 24, line 26, through page 25, line 8, with the following rewritten paragraph:

Search of the Genbank database (Boguski, et al., *Science* 265:1993-1994, 1994) of expressed sequence tag (dbEST) using the maize form-I LPAAT protein (Brown, et al., *Plant Mol. Biol.* 26: 211-223, 1994) sequences as probes resulted in the identification of several short stretches of human cDNA sequences with homology to the maize LPAAT protein sequence. These cDNA sequences of interest were derived from single-run partial sequencing of random human cDNA clones projects carried out mainly by I.M.A.G.E. Consortium [LLNL] cDNA clones program. An example of the amino acids sequence homology between the maize LPAAT and a human cDNA clone (GenBank#T55627) is shown below:

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150 GLQRLKDFPRPFWLALFVEGTRF  172 (SEQ ID NO: 28)
    :: .: :. :. :    . : .  : .  :::::
    GLRRLSDYPEYMWFLLYCEGTRF  (SEQ ID NO: 29)
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Please replace the paragraph beginning on page 25, line 18, through page 26, line 17, with the following rewritten paragraph:

Accordingly, a synthetic oligonucleotides, 5'-GACTACCCC GAGTACATGTGGTTTCTC-3' <u>(SEQ ID NO: 30)</u> (oLPTg_1F) was ordered (Life Technologies, Gaithersburg, MD) based on the coding region corresponding to amino acids DYPEYMWFL <u>(SEQ ID NO: 31)</u> of clone GenBank#T55627. oLPTg_1F was used in combination with a reverse vector primer (o.sport.1R), 5'-CTAGCTTATA ATACGACTCA C-3'<u>(SEQ ID NO: 32)</u>, for amplification of the 3'-region of potential LPAAT sequences from a pCMV.SPORT human leukocyte cDNA library (Life Technologies, Gaithersburg, MD). A 1,000 bp PCR fragment derived from o.sport.1R and oLPTg_1F amplification was cut with Xho I before inserting in between the Sma I and Xho I of pBluescript(II)SK(-) (Stratagene,

LaJolla, CA) to generate the plasmid pLPTγ_3'. Nucleotide sequencing (performed by the Seattle Biomedical Research Institute sequencing service) analysis of the cDNA inserts from plasmid pLPTg_3' showed it contained sequences that matched with the clone GenBank#T55627 as well as extensive homology with the C-terminal end of the maize LPAAT amino acids sequence (Brown, et al., *Plant Mol. Biol.* 26: 211-223, 1994). To isolate the 5'-portion of this putative LPAAT clone, a synthetic oligonucleotide, 5'-CACATGTCCGCCTCGTACTT CTTC-3' (SEQ ID NO: 44) (oLPTg_1R), complementary to a region just downstream of the Bam HI site of the cDNA within generate the plasmid pLPTg_3' was used in combination with a forward vector primer (o.sport.1), 5'-GACTCTAGCCTAGGCTTTTG C-3' (SEQ ID NO: 45) for amplification of the 5'-region from a pCMV.SPORT human leukocyte cDNA library (Life Technologies, Gaithersburg, MD). The PCR fragments generated were cut with Acc65 I and BamH I before inserting in between the Acc65 I and BamH I of pBluescript(II)SK(-) (Stratagene, LaJolla, CA). DNA sequence analysis of two cDNA clones containing, respectively, a 980 bp and a 770 bp Acc65 I – BamH I inserts showed they contained sequences that overlapped with the cDNA insert of pLPTγ_3' as well as extensive homology with the N-terminal end of the maize LPAAT amino acids sequence. The DNA sequence of these two cDNA clones diverged at the 5'-regions, suggesting the presence of two alternatively spliced variants with one variant (pLPγ1_5') containing an additional 62 amino acids at the N-terminus relative to the other one (pLPγ2_5'). To assemble the two halves of each cDNA into full-length clones, the 980 bp Acc65I – BamH I fragment from pLPγ1_5' or the 770 bp Acc65I – BamH I fragment from pLPγ2_5' were inserted into the Acc65I/Xho I vector prepared from pBluescript(II)SK(-) (Stratagene, LaJolla, CA) along with the 870 bp Bam HI – Xho I fragment from pLPTγ_3' via a three-part ligation to generate pSK_Lpγ1 and pSK_Lpγ2, respectively.

Please replace the paragraph on page 27, lines 3-13, with the following rewritten paragraph:

The sequence of the 376 amino acid open reading frame of hLPAATγ1 (Figure 9) was used as the query sequence to search for homologous sequences in protein databases. Search

of the Genbank database from the National Center for Biotechnology Information (NCBI) using the tblastn program showed that this protein was distinct but homologous to a human EST sequence with GenBank #H18562. Shown below is the amino acid sequences alignment of LPAAT-γ1 with this putative human LPAAT coding sequence (LPAAT-δ): (SEQ ID NOS 33 & 34)

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LPAAT-γ1    MGLLAFLKTQFVLHLLVGFVFVVSGLVINFVQLCTLALWPVSKQLY 46
              : :   ::.::. ::.   .::. :::.:: .:: :: :::. :::.
LPAAT-δ      MDLAGLLKSQFLCHLVFCYVFIASGLIINTIQLFTLLLWPINKQLF 340
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Please replace the paragraph on page 29, lines 11-28, with the following rewritten paragraph:

This example illustrates a group of experiments to see if overexpression of this human LPAATα would have any effect on mammalian cells. The entire cDNA insert (~2,300 bp) from pZplat.11 was cleaved with Asp718 I and Xho I for insertion into the mammalian expression vector pCE9 to generate pCE9.LPAAT1. pCE9 was derived from pCE2 with two modifications. The 550 bp BstY I fragment within the elongation factor-1a (EF-1a) intron of pCE2 was deleted. The multiple cloning region of pCE2 between the Asp718 I and BamH I site was replaced with the multiple cloning region spanning the Asp718 I and Bgl II sites from pLitmus28. The plasmid pCE2 was derived from pREP7b (Leung, et al., *Proc. Natl. Acad. Sci. USA*, 92: 4813-4817, 1995) with the RSV promoter region replaced by the CMV enhancer and the elongation factor-1a (EF-1a) promoter and intron. The CMV enhancer came from a 380 bp Xba I-Sph I fragment produced by PCR from pCEP4 (Invitrogen, San Diego, CA) using the primers 5'-GGCTCTAGAT ATTAATAGTA ATCAATTAC-3' (SEQ ID NO: 35) and 5'-CCTCACGCAT GCACCATGGT AATAGC-3' (SEQ ID NO: 36). The EF-1a promoter and intron (Uetsuki, et al., *J. Biol. Chem.,* 264: 5791-5798, 1989) came from a 1200 bp Sph I-Asp718 I fragment produced by PCR from human genomic DNA using the primers 5'-GGTGCATGCG TGAGGCTCCG GTGC-3' (SEQ ID NO: 37) and 5'-GTAGTTTTCA CGGTACCTGA AATGGAAG-3' (SEQ ID NO: 38). These 2 fragments were ligated into a Xba I/Asp718 I digested vector derived from pREP7b to generate pCE2.

Please replace the paragraph on page 32, lines 10-20, with the following rewritten paragraph:

Construction of pC9LPTγ1 and pC2LPTδ: The primers 5'-ggcccggtacc ATGGGCCTG CTGGCCTTC C-3' <u>(SEQ ID NO: 39)</u> (oLPγ1_1F) and 5'-taactcCTCGAG TTATTCCTT TTTCTTAAA CTC-3' <u>(SEQ ID NO: 40)</u> (oLPγ1_1R) were used to amplify the 1100 bp Acc65I – XhoI fragment by PCR from the template pSK_LPg1. The fragment generated was then inserted into a Acc65I / Xho I digested pCE9 (West, et al., *DNA Cell Biol.* 6: 691-701, 1997) expression vector to make pC9LPTγ1. Similarly, the primers 5'-atggtggtaccacc ATGGACCTC GCGGGACTG CTG-3' <u>(SEQ ID NO: 41)</u> (oLPTδ_1F) and 5'-GGAgGATATctAGAgGCCAC CAGTTC-3' <u>(SEQ ID NO: 42)</u> (oLPTδ_1R) were used to amplify the 1100 bp Acc65 I-Xba I fragment by PCR from the template #H18562. The fragment generated was then inserted into a Acc65I / Nhe I digested pCE2 (West, et al., *DNA Cell Biol.* 6: 691-701, 1997) expression vector to make pC2LPTδ.